The Patent Office
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Signed P. Mahoney

Dated 10 February 2004

Patents Form 1/77

Patents Act 1977
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THE PATENT OFFICE
23 JAN 2003
NEWPORT

23JAN03 E779426-1 C02885
P01/7700 0.00-0301554.2

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

MNL6

2. Patent application number
(The Patent Office will fill in this part)

23 JAN 2003 0301554.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

MOLECULARNATURE LIMITED
12 CRAUFURD RISE
MAIDENHEAD
BERKSHIRE
UK SL6 7LS

08337255001
Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

IMMUNOSTIMULATORY COMPOSITIONS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

DR. VINCENT A. PRICE
CARR HOUSE
KIRKLEES HALL
CLIFTON
WEST YORKSHIRE
HD6 4HD

08337248001
Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	28 —
Claim *(s)*	7 /
Abstract	0
Drawing *(s)*	2 only

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature  Date 22ND JAN '0

12. Name and daytime telephone number of person to contact in the United Kingdom DR. V.A.PRICE 01484 710 970

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

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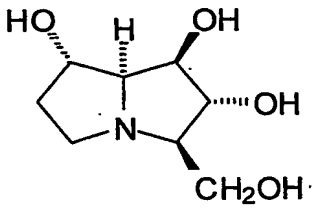
IMMUNOSTIMULATORY COMPOSITIONS

Field of the Invention

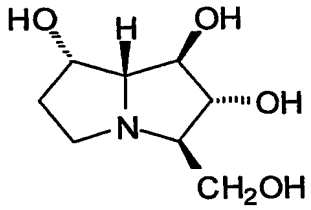
The present invention relates to immunomodulatory polyhydroxylated pyrrolizidine alkaloids and to their use in medicine. In particular, the invention relates to the use of casuarine and certain casuarine analogues as immunostimulatory drugs.

Background to the Invention

Both natural and synthetic mono- and bi-cyclic nitrogen analogues of carbohydrates are known to have potential as chemotherapeutic agents. Alexine (1) and australine (2) were the first pyrrolizidine alkaloids to be isolated with a carbon substituent at C-3, rather than the more common C-1 substituents characteristic of the necine family of pyrrolizidines.

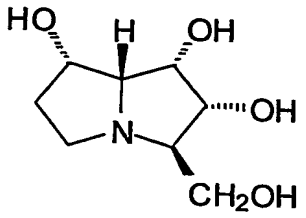


Alexine (1)



Australine (2)

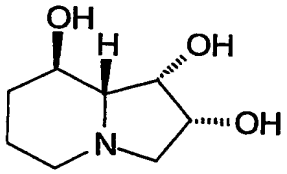
The alexines occur in all species of the genus *Alexa* and also in the related species *Castanospermum australe*. Stereoisomers of alexine, including 1,7a-diepialexine (3), have also been isolated (Nash *et al.* (1990) Phytochemistry (29) 111) and synthesised (Choi *et al.* (1991) Tetrahedron Letters (32) 5517 and Denmark and Cottell (2001) J. Org. Chem. (66) 4276-4284).



1,7a-diepialexine (3)

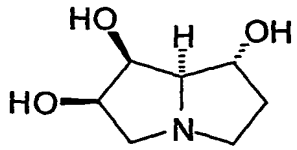
Because of the reported weak *in vitro* antiviral properties of one 7,7a-diepialexine (subsequently defined as 1,7a-diepialexine), there has been some interest in the isolation of the natural products and the synthesis of synthetic analogues.

As an indolizidine alkaloid (and so structurally distinct from the pyrrolizidine alexines), swainsonine (4) is a potent and specific inhibitor of α-mannosidase and is reported to have potential as an antimetastic, tumour anti-proliferative and immunoregulatory agent (see e.g. US5650413, WO00/37465, WO93/09117).

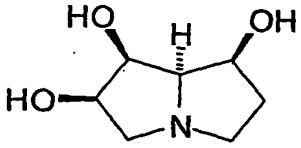


Swainsonine (4)

The effect of variation in the size of the six-membered ring of swainsonine on its glycosidase inhibitory activity has been studied: pyrrolizidine derivatives (so-called "ring contracted swainsonines") have been synthesised. However, these synthetic derivatives (1S, 2R, 7R, 7aR)-1,2,7-trihydroxypyrrolizidine (5) and the 7S-epimer (6)) were shown to have much weaker inhibitory activity relative to swainsonine itself (see US5075457).

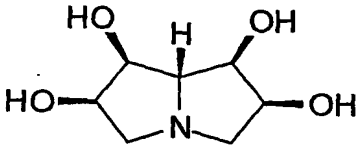


1S, 2R, 7R, 7aR)-1,2,7-trihydroxypyrrolizidine (5)



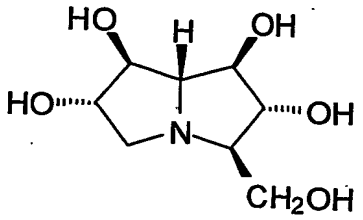
7S-epimer (6)

Another compound, 1α,2α,6α,7α,7αβ-1,2,6,7-tetrahydroxypyrrolizidine (7) is an analogue of 1,8-diepiswainsonine and described as a useful inhibitor of glycosidase enzymes in EP0417059.



1α,2α,6α,7α,7αβ-1,2,6,7-tetrahydroxypyrrolizidine (7)

Casuarine, (1R,2R,3R,6S,7S,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine (8) is a highly oxygenated bicyclic pyrrolizidine alkaloid that can be regarded as a more highly oxygenated analogue of the 1,7a-diepialexine (shown in 3) or as a C(3) hydroxymethyl-substituted analogue of the 1α,2α,6α,7α,7αβ-1,2,6,7-tetrahydroxypyrrolizidine (shown in 7).

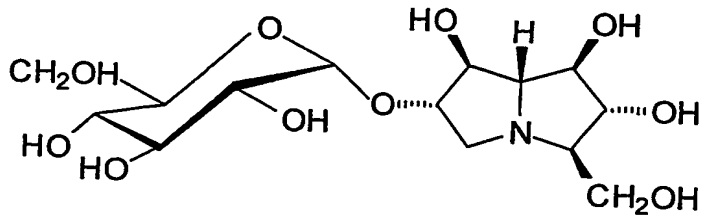


Casuarine (8)

Casuarine can be isolated from several botanical sources, including the bark of *Casuarina equisetifolia* (Casuarinaceae), the leaves and bark of *Eugenia jambolana* (Myrtaceae) and *Syzygium guineense* (Myrtaceae) (see e.g. Nash *et al.* (1994) Tetrahedron Letters (35) 7849-7852). Epimers of casuarine, and probably casuarine itself, can be synthesised by sodium hydrogen telluride-induced cyclisation of azidodimesylates (Bell *et al.* (1997) Tetrahedron Letters (38) 5869-5872).

Casuarina equisetifolia wood, bark and leaves have been claimed to be useful against diarrhoea, dysentery and colic (Chopra *et al.* (1956) Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research (India), New Delhi, p. 55) and a sample of bark has recently been prescribed in Western Samoa for the treatment of breast cancer. An African plant containing casuarine (identified as *Syzygium guineense*) has been reported to be beneficial in the treatment of AIDS patients (see Wormald *et al.* (1996) Carbohydrate Letters (2) 169-174).

The casuarine-6-α-glucoside (casuarine-6-α-D-glucopyranose, 9) has also been isolated from the bark and leaves of *Eugenia jambolana* (Wormald *et al.* (1996) Carbohydrate Letters (2) 169-174).



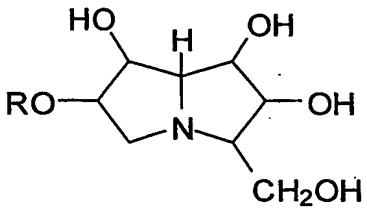
Casuarine-6-α-D-glucopyranose (9)

Eugenia jambolana is a well known tree in India for the therapeutic value of its seeds, leaves and fruit against diabetes and bacterial infections. Its fruit have been shown to reduce blood sugar levels in humans and aqueous extracts of the bark are claimed to affect glycogenolysis and glycogen storage in animals (Wormald et al. (1996) Carbohydrate Letters (2) 169-174).

The present inventors have now surprisingly discovered that casuarine and certain casuarine analogues have unexpected immunostimulatory activity, and that this activity may not be dependent on glycosidase inhibition.

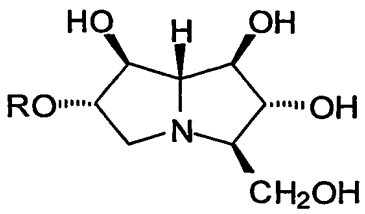
Summary of the Invention

According to the invention there is provided an isolated immunomodulatory (e.g. immunostimulatory) polyhydroxylated pyrrolizidine alkaloid for use in therapy or prophylaxis having the formula:



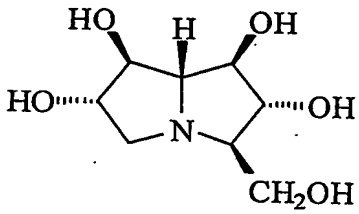
wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

The alkaloid of the invention preferably has the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

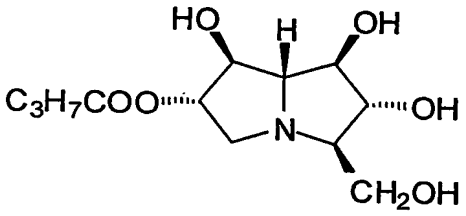
Particularly preferred is 1R,2R,3R,6S,7S,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine (casuarine), wherein R is hydrogen and which having the formula:



or a pharmaceutically acceptable derivative or salt thereof.

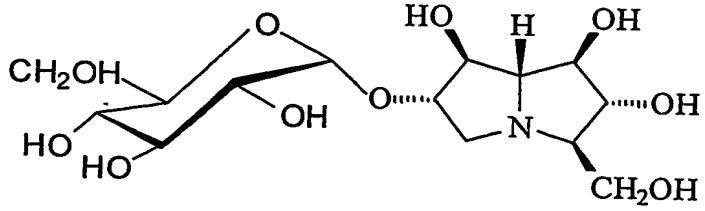
Particularly preferred is a casuarine glucoside, or a pharmaceutically acceptable salt or derivative thereof.

Other preferred alkaloids include 6-*O*-butanoylcasuarine of the formula:



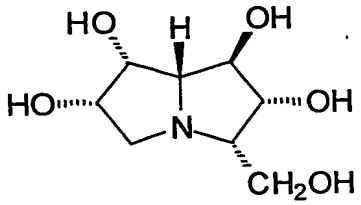
or a pharmaceutically acceptable salt or derivative thereof.

A particularly preferred casuarine glucoside is casuarine-6-α-D-glucoside of the formula:

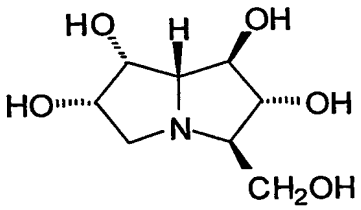


or a pharmaceutically acceptable salt or derivative thereof.

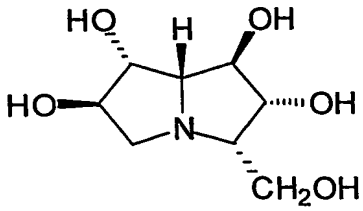
As mentioned *infra*, the invention contemplates diastereomers of the alkaloids of the invention. Particularly preferred are diastereomers selected from 3,7-*diepi*-casuarine (10), 7-*epi*-casuarine (11), 3,6,7-*triepi*-casuarine (12), 6,7-*diepi*-casuarine (13) and 3-*epi*-casuarine (14), as well as pharmaceutically acceptable salts and derivatives thereof.



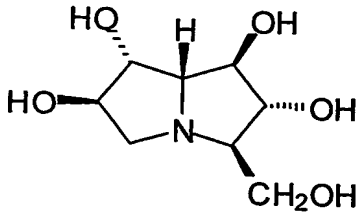
3,7-diepi-casuarine (10)



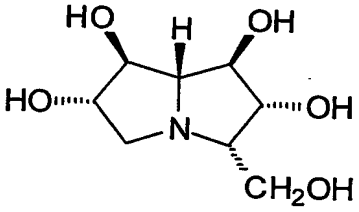
7-epicasuarine (11)



3,6,7-triepi-casuarine (12)

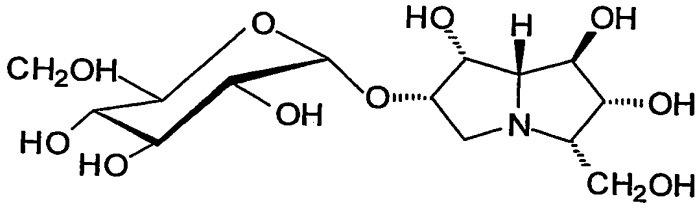


6,7-diepi-casuarine (13)

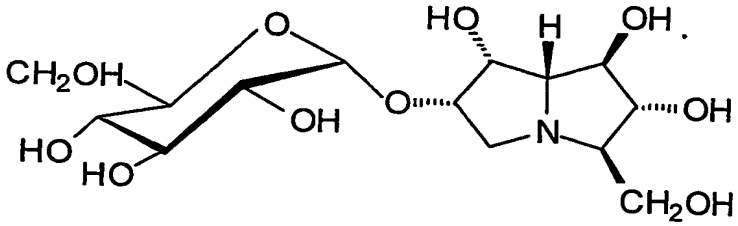


3-epi-casuarine (14)

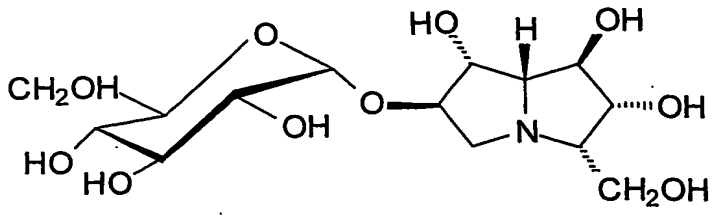
Other preferred diastereomers are selected from 3,7-*diepi*-casuarine-6-α-D-glucoside (15), 7-*epi*-casuarine-6-α-D-glucoside (16), 3,6,7-*triepi*-casuarine-6-α-D-glucoside (17), 6,7-*diepi*-casuarine-6-α-D-glucoside (18) and 3-*epi*-casuarine-6-α-D-glucoside (19), as well as pharmaceutically acceptable salts and derivatives thereof.



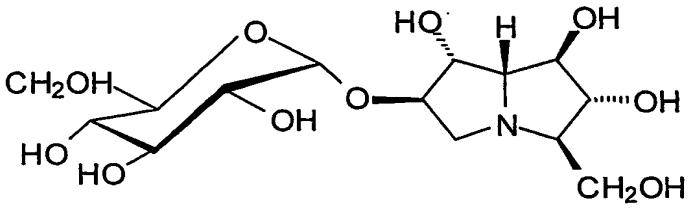
3,7-diepi-casuarine-6-α-D-glucoside (15)



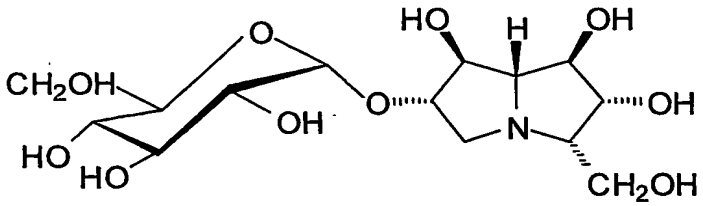
7-epi-casuarine-6-α-D-glucoside (16)



3,6,7-triepi-casuarine-6-α-D-glucoside (17)



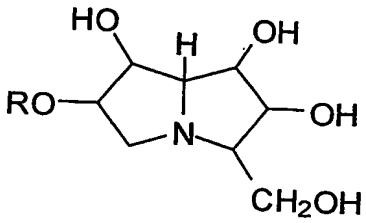
6,7-diepi-casuarine-6-α-D-glucoside (18)



3-epi-casuarine-6-α-D-glucoside (19)

Other preferred diastereomers include 7a epimers selected from 3,7,7a-tri*epi*-casuarine, 7,7a-di*epi*-casuarine, 3,6,7,7a-*tetraepi*-casuarine, 6,7,7a-tri*epi*-casuarine and 3,7a-di*epi*-casuarine, as well as pharmaceutically acceptable salts and derivatives thereof.

In another aspect the invention provides a method for immunomodulation (e.g. immunostimulation) comprising administering to a patient a composition comprising a polyhydroxylated pyrrolizidine alkaloid having the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

The immunostimulatory methods of the invention are described in more detail *infra*.

In another aspect, the invention provides a method for chemoprotection comprising administering the alkaloid of the invention to a patient undergoing chemotherapy.

The invention also contemplates the use of the polyhydroxylated pyrrolizidine alkaloid of the invention for the manufacture of a medicament for use in immunostimulation and/or chemoprotection, as well as a process for the manufacture of a medicament for use in immunostimulation and/or chemoprotection, characterized in the use of the polyhydroxylated pyrrolizidine alkaloid of the invention.

In another aspect, the invention contemplates a composition comprising the polyhydroxylated pyrrolizidine alkaloid of the invention in combination with an immunostimulant and/or cytotoxic agent (e.g. AZT) and/or an antimicrobial (e.g. antibacterial) agent and/or an antiviral agent. Such compositions preferably further comprise a pharmaceutically acceptable excipient.

In another aspect the invention contemplates a vaccine comprising the polyhydroxylated pyrrolizidine alkaloid of the invention in combination with an antigen, the alkaloid being present in an amount sufficient to produce an adjuvant effect on vaccination.

In another aspect the invention contemplates a pharmaceutical kit of parts comprising the polyhydroxylated pyrrolizidine alkaloid of the invention in combination with an immunostimulant and/or cytotoxic agent (e.g. AZT) and/or an antimicrobial (e.g. antibacterial) agent and/or an antiviral agent. Such kits preferably further comprise instructions for use in immunotherapy.

Detailed Description of the Invention

Definitions

Where used herein and unless specifically indicated otherwise, the following terms are intended to have the following meanings in addition to any broader (or narrower) meanings the terms might enjoy in the art:

The term *adjunctive* (as applied to the use of the drugs of the invention in therapy) defines uses in which the pyrrolizidine alkaloid is administered together with one or more other drugs, interventions, regimens or treatments (such as surgery and/or irradiation). Such adjunctive therapies may comprise the concurrent, separate or sequential administration/application of the pyrrolizidine alkaloid of the invention and the other treatment(s). Thus, in some embodiments, adjunctive use of the pyrrolizidine alkaloid of the invention is reflected in the formulation of the pharmaceutical compositions of the invention. For example, adjunctive use may be reflected in a specific unit dosage, or in formulations in which the pyrrolizidine alkaloid of the invention is present in admixture with the other drug(s) with which it is to be used adjunctively (or else physically associated with the other drug(s) within a single unit dose). In other embodiments, adjunctive use of the pyrrolizidine alkaloid of the invention may be reflected in the composition of the pharmaceutical kits of the invention, wherein the pyrrolizidine alkaloid of the invention is co-packaged (e.g. as part of an array of unit doses) with the other drug(s) with which it is to be used adjunctively. In yet other embodiments, adjunctive use of the pyrrolizidine alkaloid of the invention may be reflected in the content of the information and/or instructions co-packaged with the pyrrolizidine alkaloid relating to formulation and/or posology.

The term *herbal medicine* is used herein to define a pharmaceutical composition in which at least one active principle is not chemically synthesized and is a phytochemical constituent of a plant. In most cases, this non-synthetic active principle is not isolated (as defined herein), but present together with other phytochemicals with which it is associated in the source plant. In some cases, however, the plant-derived *bioactive principle(s)* may be in a concentrated fraction or isolated (sometimes involving high degrees of purification). In many cases, however, the herbal medicine comprises a more or less crude extract, infusion or fraction of a plant or even an unprocessed whole plant (or part thereof), though in such cases the plant (or plant part) is usually at least dried and/or milled.

The term *bioactive principle* is used herein to define a phytochemical which is necessary or sufficient for the pharmaceutical efficacy of the herbal medicament in which it is comprised. In the case of the present invention, the bioactive principle comprises the immunostimulatory alkaloid of the invention (e.g. casuarine, casuarine glucoside or mixtures thereof).

The term *standard specification* is used herein to define a characteristic, or a phytochemical profile, which is correlated with an acceptable quality of the herbal medicine. In this context, the term *quality* is used to define the overall fitness of the herbal medicament for its intended use, and includes the presence of one or more of the bioactive principles (at an appropriate concentration) described above or else the presence of one or more bioactive markers or a phytochemical profile which correlates with the presence of one or more of the bioactive principles (at an appropriate concentration).

The term *phytochemical profile* is used herein to define a set of characteristics relating to different phytochemical constituents.

The term *isolated* as applied to the pyrrolizidine alkaloids of the invention is used herein to indicate that the alkaloid exists in a physical milieu distinct from that in which it occurs in nature. For example, the isolated material may be substantially isolated (for example purified) with respect to the complex cellular milieu in which it naturally occurs. When the isolated material is purified, the absolute level of purity is not critical and those skilled in the art can readily determine appropriate levels of purity according to the use to which the material is to be put. Preferred, however, are purity levels of 90% w/w, 99% w/w or higher. In some circumstances, the isolated alkaloid forms part of a composition (for example a more or less crude extract containing many other substances) or buffer system, which may for example contain other components. In other circumstances, the isolated alkaloid may be purified to essential homogeneity, for example as determined spectrophotometrically, by NMR or by chromatography (for example GC-MS).

The term *pharmaceutically acceptable derivative* as applied to the pyrrolizidine alkaloids of the invention define alkaloids which are obtained (or obtainable) by chemical derivatization of the parent pyrrolizidine alkaloids of the invention. The pharmaceutically acceptable derivatives are therefore suitable for administration to or use in contact with the tissues of humans without undue toxicity, irritation or allergic response (i.e. commensurate with a reasonable benefit/risk ratio). Preferred derivatives are those

obtained (or obtainable) by alkylation, esterification or acylation of the parent pyrrolizidine alkaloids of the invention. The derivatives may be immunostimulatory *per se*, or may be inactive until processed *in vivo*. In the latter case, the derivatives of the invention act as pro-drugs. Particularly preferred pro-drugs are ester derivatives which are esterified at one or more of the free hydroxyls and which are activated by hydrolysis *in vivo*. The pharmaceutically acceptable derivatives of the invention retain some or all of the immunostimulatory activity of the parent alkaloid. In some cases, the immunostimulatory activity is increased by derivatization. Derivatization may also augment other biological activities of the alkaloid, for example bioavailability and/or glycosidase inhibitory activity and/or glycosidase inhibitory profile. For example, derivatization may increase glycosidase inhibitory potency and/or specificity.

The term *pharmaceutically acceptable salt* as applied to the pyrrolizidine alkaloids of the invention defines any non-toxic organic or inorganic acid addition salt of the free base compounds which are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and which are commensurate with a reasonable benefit/risk ratio. Suitable pharmaceutically acceptable salts are well known in the art. Examples are the salts with inorganic acids (for example hydrochloric, hydrobromic, sulphuric and phosphoric acids), organic carboxylic acids (for example acetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, dihydroxymaleic, benzoic, phenylacetic, 4-aminobenzoic, 4-hydroxybenzoic, anthranilic, cinnamic, salicylic, 2-phenoxybenzoic, 2-acetoxybenzoic and mandelic acid) and organic sulfonic acids (for example methanesulfonic acid and p-toluenesulfonic acid). The alkaloid drugs of the invention may also be converted into salts by reaction with an alkali metal halide, for example sodium chloride, sodium iodide or lithium iodide. Preferably, the pyrrolizidine alkaloids of the invention are converted into their salts by reaction with a stoichiometric amount of sodium chloride in the presence of a solvent such as acetone.

These salts and the free base compounds can exist in either a hydrated or a substantially anhydrous form. Crystalline forms of the compounds of the invention are also contemplated and in general the acid addition salts of the pyrrolizidine alkaloids of the invention are crystalline materials which are soluble in water and various hydrophilic organic solvents and which in comparison to their free base forms, demonstrate higher melting points and an increased solubility.

In its broadest aspect, the present invention contemplates all optical isomers, racemic forms and diastereomers of the pyrrolizidine alkaloids of the invention. Those skilled in the art will appreciate that, owing to the asymmetrically substituted carbon atoms present in the alkaloids of the invention, the pyrrolizidine alkaloids of the invention may exist and be synthesised and/or isolated in optically active and racemic forms. Thus, references to the pyrrolizidine alkaloids of the present invention encompass the pyrrolizidine alkaloids as a mixture of diastereomers, as individual diastereomers, as a mixture of enantiomers as well as in the form of individual enantiomers.

Therefore, the present invention contemplates all optical isomers and racemic forms thereof of the alkaloids of the invention, and unless indicated otherwise (e.g. by use of dash-wedge structural formulae) the compounds shown herein are intended to encompass all possible optical isomers of the compounds so depicted. In cases where the stereochemical form of the alkaloid is important for pharmaceutical utility, the invention contemplates use of an isolated eutomer.

Biological activities of the alkaloids of the invention

Without wishing to be bound by any theory, it is thought that the immunomodulatory activity of the alkaloids of the invention may arise from the stimulation of cytokine secretion *in vivo*. In particular, it is thought that that the immunostimulatory activity of the alkaloids of the invention arises from the stimulation of secretion of one or more cytokines, including interleukin 12 (IL-12).

IL-12 is the primary mediator of type-1 immunity. It induces NK cells to produce IFN-γ as part of the innate immune response and promotes the expansion of CD4 Th1 cells and cytotoxic CD8 cells which produce IFN-γ. It therefore increases T-cell invasion of tumours as well as the susceptibility of tumour cells to T-cell invasion.

Thus, the alkaloids of the invention are preferably stimulators of cytokine secretion. Particularly preferred are alkaloids which induce, potentiate, activate or stimulate the release one or more cytokines (for example IL-12, optionally together with one or more other cytokines) *in vivo*.

This primary immunostimulatory activity of the alkaloids of the invention is particularly important in certain medical applications (discussed in detail *infra*). For example,

increased production of IL-12 may overcome the suppression of innate and cellular immunities of HIV-1-infected individuals and AIDS patients.

Without wishing to be bound by any theory, it is thought that at least some of the pharmacological activities of the alkaloids of the invention may be based on a secondary glycosidase inhibitory activity.

Such glycosidase inhibition may lead to any or all of the following *in vivo*:

- Modification of tumour cell glycosylation (e.g. tumour antigen glycosylation);
- Modification of viral protein glycosylation (e.g. virion antigen glycosylation);
- Modification of cell-surface protein glycosylation in infected host cells;
- Modification of bacterial cell walls.

This ancillary biological activity may therefore augment the primary immunostimulatory activity in some preferred embodiments of the invention. It may be particularly desirable in certain medical applications, including the treatment of proliferative disorders (such as cancer) or in applications where infection is attendant on immune suppression. For example, selective modification of virion antigen glycosylation may render an infecting virus less (or non-) infective and/or more susceptible to endogenous immune responses. In particular, the alkaloids of the invention may alter the HIV viral envelope glycoprotein gp120 glycosylation patterns, hence inhibiting the entry of HIV into the host cell by interfering with the binding to cell surface receptors.

Thus, the alkaloids of the invention are preferably (but not necessarily) glycosidase inhibitors. Particularly preferred are alkaloids which exhibit specificity of glycosidase inhibition, for example Glucosidase I rather than mannosidases. Such preferred alkaloids can therefore be quite different in their glycosidase inhibitory profile to swainsonine and its analogues, since the latter are potent and specific inhibitors of mannosidase.

Medical applications

The invention finds application in medicine, for example in methods of therapy, prophylaxis and/or diagnosis.

The medical applications may be applied to any warm-blooded animal, including humans. The applications include veterinary applications, wherein the pyrrolizidine alkaloids of the invention are administered to non-human animals, including primates, dogs, cats, horses, cattle and sheep.

The pyrrolizidine alkaloids of the invention are immunomodulators. Thus, they find general application in the treatment or prophylaxis of conditions in which stimulation, augmentation or induction of the immune system is indicated.

Particular medical uses of the pyrrolizidine alkaloids of the invention are described in detail below.

1. Haemorestoration

The pyrrolizidine alkaloids of the invention increase splenic and bone marrow cell proliferation and can act as myeloproliferative agents. They therefore find application as haemorestoratives.

Haemorestoration may be indicated following chemotherapy (including treatment with both cycle-specific and non-specific chemotherapeutic agents), steroid administration or other forms of surgical or medical intervention (including radiotherapy). Thus, the use of the pyrrolizidine alkaloids of the invention as haemorestoratives may be adjunctive to other treatments which tend to depress splenic and bone marrow cell populations. Particularly preferred adjunctive therapies according to the invention include the administration of an immunorestorative dose of the pyrrolizidine alkaloid of the invention adjunctive to: (a) chemotherapy; and/or (b) radiotherapy; and/or (c) bone marrow transplantation; and/or (d) haemoablative immunotherapy.

2. Alleviation of immunosuppression

The pyrrolizidine alkaloids of the invention may be used to alleviate, control or modify states in which the immune system is partially or completely suppressed or depressed. Such states may arise from congenital (inherited) conditions, be acquired (e.g. by infection or malignancy) or induced (e.g. deliberately as part of the management of transplants or cancers).

Thus, the pyrrolizidine alkaloids of the invention may find application as adjunctive immunostimulants in the treatment and/or management of various diseases (including certain cancers) or medical interventions (including radiotherapy, chemotherapy and cytotoxic drug administration (for example the administration of AZT, cyclophosphamide, cortisone acetate, vinblastine, vincristine, adriamycin, 6-mercaptopurine, 5-fluorouracil, mitomycin C, chloramphenicol and other steroid-based therapies). They may therefore be used as chemoprotectants in the management of various cancers and infections (including bacterial and viral infections, e.g. HIV infection).

In particular, the pyrrolizidine alkaloids of the invention may find application as immunostimulants in the treatment or management of microbial infections which are associated with immune-suppressed states, including many viral infections (including HIV infection in AIDS) and in other situations where a patient has been immunocompromised (e.g. following infection with hepatitis C, or other viruses or infectious agents including bacteria, fungi, and parasites, in patients undergoing bone marrow transplants, and in patients with chemical or tumor-induced immune suppression).

Other diseases or disorders which may give rise to an immunosupressed state treatable according to the invention include: ataxia-telangiectasia; DiGeorge syndrome; Chediak-Higashi syndrome; Job syndrome; leukocyte adhesion defects; panhypogammaglobulinemia (e.g. associated with Bruton disease or congenital agammaglobulinemia); selective deficiency of IgA; combined immunodeficiency disease; Wiscott-Aldrich syndrome and complement deficiencies. It may be associated with organ and/or tissue (e.g. bone marrow) transplantation or grafting, in which applications the pyrrolizidine alkaloids of the invention may be used adjunctively as part of an overall treatment regimen including surgery and post-operative management of immune status.

3. Cytokine stimulation

The pyrrolizidine alkaloids of the invention may be used to induce, potentiate or activate various cytokines *in vivo*, including various interleukins (including IL-12).

Accordingly, the pyrrolizidine alkaloids of the invention find general application in the treatment or prophylaxis of conditions in which the *in vivo* induction, potentiation or activation of one or more cytokines (e.g. IL-12) is indicated. Such applications may be

employed to stimulate particular elements of the cellular immunity system, including macrophages (e.g. tissue-specific macrophages), NK and LAK cells.

4. Treatment of proliferative disorders

The invention finds application in the treatment of proliferative disorders, including various cancers and cancer metastasis. For example, the pyrrolizidine alkaloids of the invention may find particular application in the treatment of leukemias, lymphomas, melanomas, adenomas, sarcomas, carcinomas of solid tissues, melanoma, pancreatic cancer, cervico-uterine cancer, cancers of the kidney, stomach, lung, ovary, rectum, breast, prostate, bowel, gastric, liver, thyroid, neck, cervix, salivary gland, leg, tongue, lip, bile duct, pelvis, mediastinum, urethra, lung, bladder, esophagus and colon, and Kaposi's Sarcoma (e.g. when associated with AIDS).

5. Use as adjuvant

The pyrrolizidine alkaloids of the invention find utility as vaccine adjuvants, in which embodiments they may promote, induce or enhance an immune response to antigens, particularly antigens having low intrinsic immunogenicity. Without wishing to be bound by any theory, the pyrrolizidine alkaloids of the invention may augment vaccine immunogenicity by stimulating cytokine release, thereby promoting T-cell help for B cell and CTL responses. They may also change glycosylation of cancer or viral antigens and increase vaccine effectiveness.

When used as adjuvant, the compounds of the invention may be administered concurrently, separately or sequentially with administration of the vaccine. Thus, in some embodiments, the pyrrolizidine alkaloid of the invention may be present in admixture with other vaccine component(s), or else co-packaged (e.g. as part of an array of unit doses) with the other vaccine components with which it is to be used as adjuvant. In yet other embodiments, the use of the pyrrolizidine alkaloids of the invention as adjuvant is simply reflected in the content of the information and/or instructions co-packaged with the vaccine components and relating to the vaccination procedure, vaccine formulation and/or posology.

Posology

The pyrrolizidine alkaloids of the present invention can be administered by oral or

parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration.

The amount of the pyrrolizidine alkaloid administered can vary widely according to the particular dosage unit employed, the period of treatment, the age and sex of the patient treated, the nature and extent of the disorder treated, and the particular pyrrolizidine alkaloid selected.

Moreover, the pyrrolizidine alkaloids of the invention can be used in conjunction with other agents known to be useful in the treatment of diseases, disorders or infections where immunostimulation is indicated (as described *infra*) and in such embodiments the dose may be adjusted accordingly.

In general, the effective amount of the pyrrolizidine alkaloid administered will generally range from about 0.01 mg/kg to 500 mg/kg daily. A unit dosage may contain from 0.05 to 500 mg of the pyrrolizidine alkaloid, and can be taken one or more times per day. The pyrrolizidine alkaloid can be administered with a pharmaceutical carrier using conventional dosage unit forms either orally, parenterally, or topically, as described below.

The preferred route of administration is oral administration. In general a suitable dose will be in the range of 0.01 to 500 mg per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 50 mg per kilogram body weight per day and most preferably in the range 1 to 5 mg per kilogram body weight per day.

The desired dose is preferably presented as a single dose for daily administration. However, two, three, four, five or six or more sub-doses administered at appropriate intervals throughout the day may also be employed. These sub-doses may be administered in unit dosage forms, for example, containing 0.001 to 100 mg, preferably 0.01 to 10 mg, and most preferably 0.5 to 1.0 mg of active ingredient per unit dosage form.

Formulation

The compositions of the invention comprise the pyrrolizidine alkaloid of the invention, optionally together with a pharmaceutically acceptable excipient.

The pyrrolizidine alkaloid of the invention may take any form. It may be synthetic, purified or isolated from natural sources (for example from *Casuarina equisetifolia* or *Eugenia jambolana*), using techniques described in the art (and referenced *infra*).

When isolated from a natural source, the pyrrolizidine alkaloid of the invention may be purified. However, the compositions of the invention may take the form of herbal medicines, as hereinbefore defined. Such herbal medicines preferably are analysed to determine whether they meet a standard specification prior to use.

The herbal medicines for use according to the invention may be dried plant material. Alternatively, the herbal medicine may be processed plant material, the processing involving physical or chemical pre-processing, for example powdering, grinding, freezing, evaporation, filtration, pressing, spray drying, extrusion, supercritical solvent extraction and tincture production. In cases where the herbal medicine is administered or sold in the form of a whole plant (or part thereof), the plant material may be dried prior to use. Any convenient form of drying may be used, including freeze-drying, spray drying or air-drying.

In embodiments where the pyrrolizidine alkaloid of the invention is formulated together with a pharmaceutically acceptable excipient, any suitable excipient may be used, including for example inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc.

The pharmaceutical compositions may take any suitable form, and include for example tablets, elixirs, capsules, solutions, suspensions, powders, granules and aerosols.

The pharmaceutical composition may take the form of a kit of parts, which kit may comprise the composition of the invention together with instructions for use and/or a plurality of different components in unit dosage form.

Tablets for oral use may include the pyrrolizidine alkaloid of the invention, either alone or together with other plant material associated with the botanical source(s) (in the case of

herbal medicine embodiments). The tablets may contain the pyrrolizidine alkaloid of the invention mixed with pharmaceutically acceptable excipients, such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatin capsules in which the pyrrolizidine alkaloid of the invention is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity.

Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinylpyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

The compounds of the invention may also be presented as liposome formulations.

For oral administration the pyrrolizidine alkaloid of the invention can be formulated into solid or liquid preparations such as capsules, pills, tablets, troches, lozenges, melts, powders, granules, solutions, suspensions, dispersions or emulsions (which solutions, suspensions dispersions or emulsions may be aqueous or non-aqueous). The solid unit

dosage forms can be a capsule which can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, calcium phosphate, and cornstarch.

In another embodiment, the pyrrolizidine alkaloids of the invention are tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch, or gelatin, disintegrating agents intended to assist the break-up and dissolution of the tablet following administration such as potato starch, alginic acid, corn starch, and guar gum, lubricants intended to improve the flow of tablet granulations and to prevent the adhesion of tablet material to the surfaces of the tablet dies and punches, for example, talc, stearic acid, or magnesium, calcium, or zinc stearate, dyes, coloring agents, and flavoring agents intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient.

Suitable excipients for use in oral liquid dosage forms include diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptably surfactant, suspending agent or emulsifying agent.

The pyrrolizidine alkaloids of the invention may also be administered parenterally, that is, subcutaneously, intravenously, intramuscularly, or interperitoneally.

In such embodiments, the pyrrolizidine alkaloid is provided as injectable doses in a physiologically acceptable diluent together with a pharmaceutical carrier (which can be a sterile liquid or mixture of liquids). Suitable liquids include water, saline, aqueous dextrose and related sugar solutions, an alcohol (such as ethanol, isopropanol, or hexadecyl alcohol), glycols (such as propylene glycol or polyethylene glycol), glycerol ketals (such as 2,2-dimethyl-1,3-dioxolane-4-methanol), ethers (such as poly(ethylene-glycol) 400), an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant (such as a soap or a detergent), suspending agent (such as pectin, carhomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose), or emulsifying agent and other pharmaceutically adjuvants. Suitable oils which can be used in the parenteral formulations of this invention are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum, and mineral oil.

Suitable fatty acids include oleic acid, stearic acid, and isostearic acid. Suitable fatty acid esters are, for example, ethyl oleate and isopropyl myristate.

Suitable soaps include fatty alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, alkyl pyridinium halides, and alkylamines acetates; anionic detergents, for example, alkyl, aryl, and olefin sulphonates, alkyl, olefin, ether, and monoglyceride sulphates, and sulphosuccinates; nonionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers; and amphoteric detergents, for example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quarternary ammonium salts, as well as mixtures.

The parenteral compositions of this invention will typically contain from about 0.5 to about 25% by weight of the pyrrolizidine alkaloid of the invention in solution. Preservatives and buffers may also be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The pyrrolizidine alkaloids of the invention may also be administered topically, and when done so the carrier may suitably comprise a solution, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Topical formulations may contain a concentration of the alkaloid from about 0.1 to about 10% w/v (weight per unit volume).

When used adjunctively, the pyrrolizidine alkaloids of the invention may be formulated for use with one or more other drug(s). In particular, the pyrrolizidine alkaloids of the invention may be used in combination with antitumor agents, antimicrobial agents, anti-inflammatories, antiproliferative agents and/or other immunostimulatory agents. For example, the pyrrolizidine alkaloids of the invention may be used with anti-viral and/or anti-proliferative agents such as cytokines, including interleukins-2 and 12, interferons

and inducers thereof, tumor necrosis factor (TNF) and/or transforming growth factor (TGF), as well as with myelosuppressive agents and/or chemotherapeutic agents (such as doxorubicin, 5-fluorouracil, cyclophosphamide and methotrexate), isoniazid (e.g. in the prevention or treatment of peripheral neuropathy) and with analgesics (e.g. NSAIDs) for the prevention and treatment of gastroduodenal ulcers.

Thus, adjunctive use may be reflected in a specific unit dosage designed to be compatible (or to synergize) with the other drug(s), or in formulations in which the pyrrolizidine alkaloid is admixed with one or more antitumor agents, antimicrobial agents and/or antiinflammatories (or else physically associated with the other drug(s) within a single unit dose). Adjunctive uses may also be reflected in the composition of the pharmaceutical kits of the invention, in which the pyrrolizidine alkaloid of the invention is co-packaged (e.g. as part of an array of unit doses) with the antitumor agents, antimicrobial agents and/or antiinflammatories. Adjunctive use may also be reflected in information and/or instructions relating to the co-administration of the pyrrolizidine alkaloid with antitumor agents, antimicrobial agents and/or antiinflammatories.

Exemplification

The invention will now be described with reference to specific Examples. These are merely exemplary and for illustrative purposes only: they are not intended to be limiting in any way to the scope of the monopoly claimed or to the invention described. These examples constitute the best mode currently contemplated for practicing the invention.

Example 1: Induction of IL-12 secretion

Mice

BALB/c male and female mice bred and maintained at the University of Strathclyde under conventional conditions were used at 8 weeks old.

Isolation of bone marrow and culture of dendritic cells

Bone marrow was obtained from the femurs of mice. The femurs were washed in 70% ethanol and placed in a clean petri dish. Dendritic cell (DC) medium (2.5% granulocyte-macrophage colony-stimulating factor (GM-CSF), 10% heat and activated foetal calf serum, 1% L-glutamine, 1% Penicillin/Streptomycin in RPMI-1640 medium) was injected into the bone marrow of the femur by a pumping action and the cells and medium

were collected. 1ml of the cells in medium was added to a 75cm^2 flask with 15mls of DC medium. The flasks were then incubated at 37°C, 5% CO_2 to allow DC growth and development. After 5 days an additional 10mls of DC medium was added.

Harvesting of dendritic cells

After 10 days of incubation of bone marrow with GM-CSF, the dendritic cells were harvested. This process was carried out in a tissue culture hood. The contents of the flasks were poured into centrifuge tubes to ensure collection of floating DCs. Approximately 10mls of cooled phosphate buffered saline (PBS) was added to each empty flask, the flasks gently agitated and the contents collected. This ensured recovery of adhesive DCs. The collected contents of the flasks were centrifuged for 5 minutes at 200*g* and the pellet resuspended in 2mls of DC medium without GM-CSF. A cell count was then carried out.

Cell count and assay conditions

Cells were counted using a haemocytometer. Approximately 20µl of the resuspended cells was pipetted into the chamber of the haemocytometer, the cells were adjusted to the correct cell concentration (approx. 5 x 10^4, and not less than 1 x 10^4, per well) and then plated out for assay.

The plates were incubated overnight at 37°C with 5% CO_2 and allowed to settle (harvesting stimulates them). The next day the compounds/controls were added then again incubated at 37°C with 5% CO_2 for 24 hrs (or 48 hrs). Harvesting and addition of the compounds was all done in a hood. The plates were then frozen to kill the cells and once defrosted the supernatant analysed as described below.

Measurement of IL-12

Using an enzyme linked immunosorbent assay (ELISA) IL-12 concentration in the supernatants was measured. All reagents used in this assay were from PharMingen. A 96-well flat-bottomed ELISA plate was coated with purified rat anti-mouse IL-12 (p40/p70) MAb (Cat no. 554478) at 2µg/ml diluted in PBS pH 9.0 at 50µl/well. The plate was then covered in cling film and incubated at 4°C. Following incubation the plate was washed 3 times in washing buffer and dried. 200µl of blocking buffer (10% foetal calf serum in PBS pH 7.0) was added to each well then covered in cling film and incubated at

37°C for 45 minutes. The plate was washed 3 times and dried. Recombinant mouse Il-12 standard was added at 30µl in duplicate wells, starting at 10ng/ml then 5, 2.5, 1.25, 0.625, 0.31, 0.156, 0.078, 0.039, 0.020, 0.010, 0.005ng/ml. Standards were diluted in blocking buffer. The supernatant samples were added in at 50µl/well. The plate was then covered in cling film and incubated for 2 hours at 37°C. The plate was then washed 4 times, dried and the secondary antibody added.

Biotin labelled anti-mouse IL-12 (p40/p70) MAb (Cat no. 18482D) at 1µg/ml (diluted in blocking buffer) was added to each well at a volume of 100µl/well. The plate was covered in cling film and incubated at 37°C for 1 hour. The plate was then washed 5 times, dried and the conjugate added. Streptavidin-AKP (Cat no. 13043E) at 100µl/well was added at a dilution of 1/2000 in blocking buffer followed by incubation under cling film at 37°C for 45 minutes.

The plate was finally washed 6 times, dried and the substrate added. pNPP (Sigma) in glycine buffer at 1mg/ml was added at 100 µl/well. The plate was then covered in tinfoil, incubated at 37°C and checked every 30 minutes for a colour change.

The plate was then read at 405nm using a SPECTRAmax 190 spectrometer. The results are shown in Figures 1 and 2, in which LPS is lipopolysaccharide, IFN-g is interferon gamma, 462a is casuarine (8), 462b is casuarine-6-α-D-glucopyranose (9), 23 is 7-epicasuarine (11) and 24 is 3,7-diepi-casuarine (10).

When tested at 0.03ng/ml in the same assay, swainsonine (4) failed to induce IL-12 secretion.

Example 2: Inhibition of glycosidase activity

Assays were carried out in microtitre plates. Enzymes were assayed in 0.1M citric acid/0.2M di-sodium hydrogen phosphate (McIlvaine) buffers at the optimum pH for the enzyme. All assays were carried out at 20°C. For screening assays the incubation assay consisted of 10 µl of enzyme solution, 10 µl of inhibitor solution (made up in water) and 50 µl of the appropriate 5 mM *p*-nitrophenyl substrate (3.57mM final conc.) made up in McIlvaine buffer at the optimum pH for the enzyme.

The reactions were stopped with 0.4M glycine (pH 10.4) during the exponential phase of the reaction, which was determined at the beginning of the assay using blanks with water, which were incubated for a range of time periods to measure the reaction rate using 5 mM substrate solution. Endpoint absorbances were read at 405nm with a Biorad microtitre plate reader (Benchmark). Water was substituted for the inhibitors in the blanks.

The enzymes tested are shown in the following table:

Enzyme	Source	pH	Conc.	Substrate
α-glucosidase	Bakers yeast	6.0	0.1 unit/ml	PNP-α-D-glucopyranoside
β-glucosidase	Almonds	5.0	0.2 unit/ml	PNP-β-D-glucopyranoside
α-galactosidase	Coffee beans	6.5	1 unit/ml	PNP-α-D-galactopyranoside
β-galactosidase	Bovine liver	7.3	0.1 unit/ml	PNP-β-D-galactopyranoside
α-mannosidase	Jack bean	4.5	0.1 unit/ml	PNP-α-D-mannopyranoside
β-N-acetyl glucosaminidase	*Aspergillus niger*	4.25	0.1 unit/ml	PNP-N-acetyl-β-D-glucosminide
Naringinase	*P. decumbens*	4.0	1 unit/ml	PNP-α-L-rhamnopyranoside

The results (% inhibition) for casuarine (8), 3,7-diepi-casuarine (10) and 7-epicasuarine (11) (all at 1mg/ml) are shown below:

Enzyme	Casuarine (8)	3,7-diepi (10)	7-epi (11)
α-glucosidase	76	-2	11
β-glucosidase	34	5	30
α-galactosidase	17	-11	1
β-galactosidase	26	24	35
β-N-acetylglucosaminidase	10	8	10
Naringinase	3	5	25
Mannosidase	12	6	-4

These results indicate a lack, or very low level, of inhibition of all the enzymes by these compounds apart from weak to moderate inhibition of α-glucosidase by casuarine (8). Swainsonine would give very high inhibition of the mannosidase at this concentration.

Example 3: Differential inhibition of mannosidase and glucosidase

The glycosidase inhibitory profiles of swainsonine (4), casuarine (8) and casuarine glucoside (9) with respect to a mannosidase and a glucosidase were compared. The results (all at <0.1mg/ml) are shown in the table below.

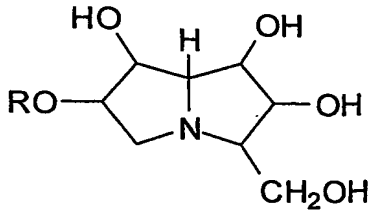
Alkaloid	Mannosidase inhibition	Glucosidase I inhibition
Swainsonine (4)	+	-
Casuarine (8)	-	+
Casuarine glucoside (9)	-	+

Equivalents

The foregoing description details presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are intended to be encompassed within the claims appended hereto.

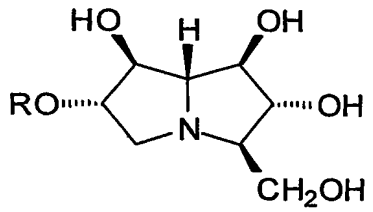
CLAIMS:

1. An isolated immunostimulatory polyhydroxylated pyrrolizidine alkaloid for use in therapy or prophylaxis having the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

2. The alkaloid of claim 1 having the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

3. The alkaloid of claim 1 or claim 2 which induces, potentiates or activates one or more cytokines *in vivo*.

4. The alkaloid of claim 3 wherein the one or more cytokines comprises one or more interleukins.

5. The alkaloid of claim 4 wherein the one or more interleukins comprises IL-12.

6. The alkaloid of any one of the preceding claims which is a glycosidase inhibitor.

7. The alkaloid of claim 6 which inhibits glucosidase.

8. The alkaloid of any one of the preceding claims which does not inhibit mannosidase.

9. The alkaloid of any one of the preceding claims which:

(a) modifies tumour cell glycosylation (e.g. tumour antigen glycosylation); and/or

(b) modifies viral protein glycosylation (e.g. virion antigen glycosylation); and/or

(c) modifies cell-surface protein glycosylation in infected host cells; and/or

(d) modifies bacterial cell walls,

when administered *in vivo*.

10. The alkaloid of any one of the preceding claims which is an acyl derivative.

11. The alkaloid of claim 10 which is:

(a) peracylated; or

(b) acylated at C-3 hydroxymethyl; or

(c) acylated at C-6;

(d) acylated at C-3 hydroxymethyl and C-6.

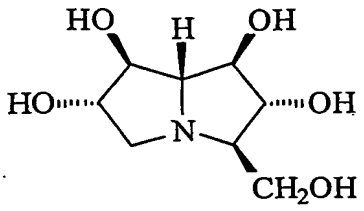
12. The alkaloid of claim 10 or claim 11 wherein the acyl derivative is alkanoyl or aroyl.

13. The alkaloid of claim 12 wherein the acyl derivative is an alkanoyl selected from acetyl, propanoyl or butanoyl.

14. The alkaloid of any one of claims 1 to 13 wherein R is a saccharide moiety.

15. The alkaloid of claim 14 wherein R is a glucoside or arabinoside moiety.

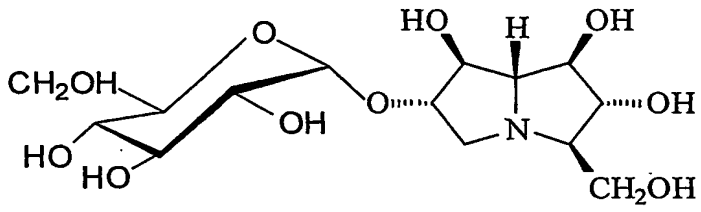
16. The alkaloid of claim 2 which is 1R,2R,3R,6S,7S,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine (casuarine), wherein R is hydrogen and having the formula:



or a pharmaceutically acceptable salt or derivative thereof.

17. The alkaloid of claim 2 which is a casuarine glycoside, or a pharmaceutically acceptable salt or derivative thereof.

18. The alkaloid of claim 17 which is casuarine-6-α-D-glucoside of the formula:



or a pharmaceutically acceptable salt or derivative thereof.

19. The alkaloid of claim 2 which is 6-*O*-butanoylcasuarine, or a pharmaceutically acceptable salt or derivative thereof.

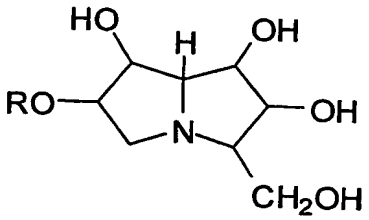
20. The alkaloid of claim 1 which is selected from:

(a) 3,7-*diepi*-casuarine;
(b) 7-*epi*-casuarine;
(c) 3,6,7-*triepi*-casuarine;
(d) 6,7-*diepi*-casuarine;
(e) 3-*epi*-casuarine;
(f) 3,7-*diepi*-casuarine-6-α-D-glucoside;
(g) 7-*epi*-casuarine-6-α-D-glucoside;
(h) 3,6,7-*triepi*-casuarine-6-α-D-glucoside;
(i) 6,7-*diepi*-casuarine-6-α-D-glucoside; and

(j) 3-*epi*-casuarine-6-α-D-glucoside,

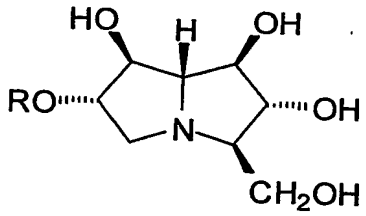
or a pharmaceutically acceptable salt or derivative thereof.

21. A method for immunostimulation comprising administering to a patient a composition comprising a polyhydroxylated pyrrolizidine alkaloid having the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

22. The method of claim 21 wherein the alkaloid has the formula:



wherein R is selected from the group comprising hydrogen, straight or branched, unsubstituted or substituted, saturated or unsaturated acyl, alkyl (e.g. cycloalkyl), alkenyl, alkynyl and aryl groups, or a pharmaceutically acceptable salt or derivative thereof.

23. The method of claim 21 or claim 22 wherein the alkaloid is as defined in any one of claims 1 to 20.

24. The method of any one of claims 21 to 23 wherein the composition comprises an isolated alkaloid or a combination of one or more of the alkaloids as defined in any one of claims (for example wherein the composition comprises a combination of casuarine and casuarine-6-α-D-glucoside).

25. The method of any one of claims 21 to 24 wherein the composition is a herbal medicine.

26. The method of claim 25 wherein the botanic source of the herbal medicine comprises one or more plant species selected from:

(a) a member of the taxon *Myrtaceae* (for example *Myrtus spp. (e.g. M. communis*), *Syzygium* spp. (e.g. *S. guineense*) or *Eugenia* spp. (e.g. *E. jambolana*); or

(b) a member of the taxon *Casuarinaceae*;

(c) a combination of two or more plant species selected from both of the taxons of (a) and (b).

27. The method of any one of claims 21 to 26 which comprises haemorestoration.

28. The method of claim 27 wherein the haemorestoration is adjunctive to:

(a) chemotherapy; and/or

(b) radiotherapy; and/or

(c) bone marrow transplantation; and/or

(d) haemoablative immunotherapy.

29. The method of any one of claims 21 to 28 which comprises the alleviation of immunosuppression.

30. The method of claim 29 wherein the immunosuppression is congenital, acquired (e.g. by infection or malignancy) or induced (e.g. deliberately as part of the management of transplants or cancers).

31. The method of any one of claims 21 to 30 which comprises induction, potentiation or activation of one or more cytokines (for example IL-12) *in vivo*.

32. The method of any one of claims 21 to 30 which comprises the treatment of proliferative disorders, for example proliferative disorders selected from cancer and cancer metastasis.

33. A method for chemoprotection comprising administering the alkaloid as defined in any one of claims 1 to 20 or composition as defined in any one of claims 21 to 32 to a patient undergoing chemotherapy.

34. Use of the polyhydroxylated pyrrolizidine alkaloid as defined in any one of claims 1 to 20 or composition as defined in any one of claims 21 to 32 for the manufacture of a medicament for use in immunostimulation and/or chemoprotection.

35. A process for the manufacture of a medicament for use in immunostimulation and/or chemoprotection, characterized in the use of the polyhydroxylated pyrrolizidine alkaloid as defined in any one of claims 1 to 20 or composition as defined in any one of claims 21 to 32.

36. The use of claim 34 or process of claim 35 wherein the immunostimulation and/or chemoprotection is as defined in any one of claims 21 to 33.

37. A composition comprising a polyhydroxylated pyrrolizidine alkaloid as defined in any one of the preceding claims in combination with:

(a) an immunostimulant; and/or

(b) a cytotoxic agent (e.g. cyclophosphamide, cortisone acetate, vinblastine, vincristine, adriamycin, 6-mercaptopurine, 5-fluorouracil, mitomycin C or chloramphenicol); and/or

(c) an antimicrobial (e.g. antibacterial) agent; and/or

(d) an antiviral agent (e.g. AZT).

38. The composition of claim 37 further comprising a pharmaceutically acceptable excipient.

39. A vaccine comprising a polyhydroxylated pyrrolizidine alkaloid as defined in any one of claims 1 to 20 or composition as defined in any one of claims 21 to 32 in combination with an antigen, the alkaloid being present in an amount sufficient to produce an adjuvant effect on vaccination.

40. A pharmaceutical kit of parts comprising a polyhydroxylated pyrrolizidine alkaloid as defined in any one of claims 1 to 20 or composition as defined in any one of claims 21 to 32 in combination with any or all of the adjunctive therapeutic agents defined in claim 37(a)-(d).

41. The kit of claim 40 further comprising instructions for use.

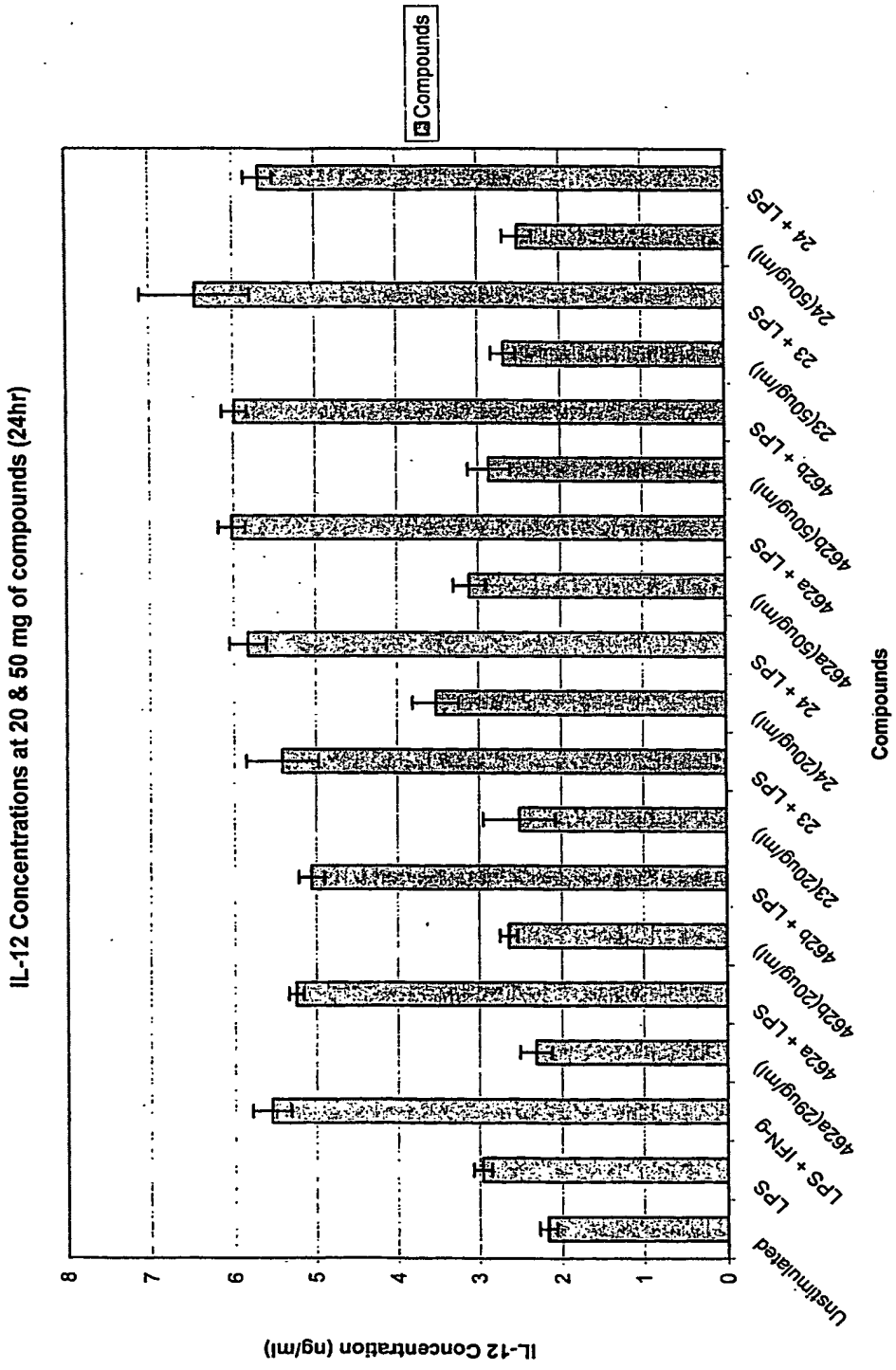

IL-12 Concentrations at 20 & 50 mg of compounds (24hr)
Compounds
IL-12 Concentration (ng/ml)
0
1
2
3
4
5
6
7
8
Unstimulated
LPS
LPS + IFN-g
462a(20ug/ml)
462a + LPS
462b(20ug/ml)
462b + LPS
23(20ug/ml)
23 + LPS
24(20ug/ml)
24 + LPS
462a(50ug/ml)
462a + LPS
462b(50ug/ml)
462b + LPS
23(50ug/ml)
23 + LPS
24(50ug/ml)
24 + LPS
Compounds

Fig. 1

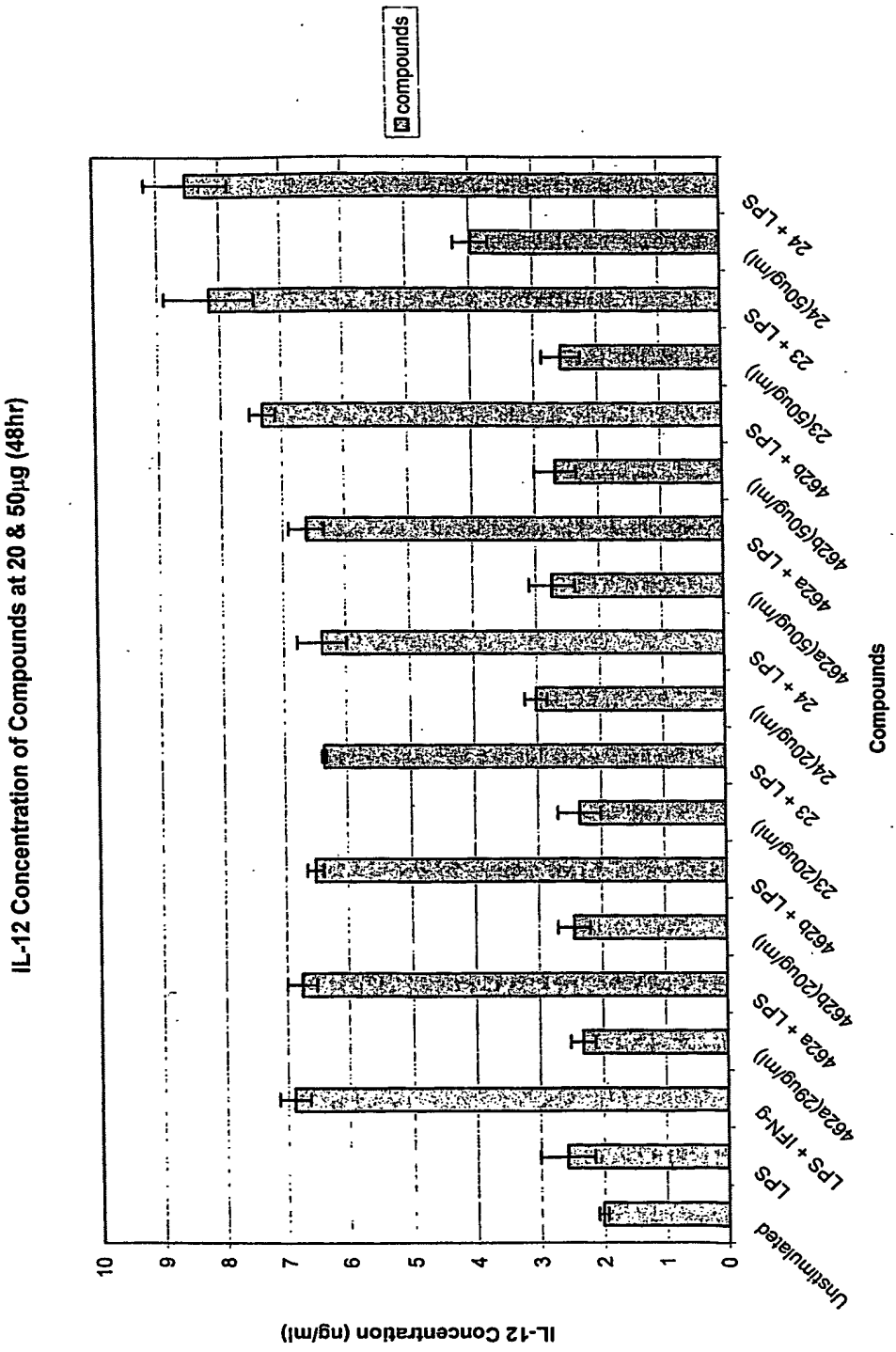

IL-12 Concentration of Compounds at 20 & 50µg (48hr)
compounds
IL-12 Concentration (ng/ml)
0
1
2
3
4
5
6
7
8
9
10
Unstimulated
LPS
LPS + IFN-g
462a(20ug/ml)
462a + LPS
462b(20ug/ml)
462b + LPS
23(20ug/ml)
23 + LPS
24(20ug/ml)
24 + LPS
462a(50ug/ml)
462a + LPS
462b(50ug/ml)
462b + LPS
23(50ug/ml)
23 + LPS
24(50ug/ml)
24 + LPS
Compounds

Fig. 2